Optimal Patient Selection for Trastuzumab Treatment in HER2-Positive Advanced Gastric Cancer

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Abstract

Purpose: Chemotherapy plus trastuzumab is standard of care for HER2-positive advanced gastric cancer (AGC). However, not all patients with HER2-positive AGC seem to benefit from trastuzumab. We evaluated the association between treatment outcomes with trastuzumab and HER2 status in patients with HER2-positive AGC.

Experimental Design: We enrolled 126 patients with HER2-positive AGC treated with trastuzumab plus chemotherapy in a training cohort. HER2 IHC (N = 126), HER2/CEP17 ratio (N = 66), and HER2 gene copy number (GCN; N = 59) were analyzed, and the optimal values for discriminating overall survival (OS) were determined using receiver operating characteristic (ROC) curve analysis. We validated the findings from the training cohort using an independent validation cohort (N = 72).

Results: Patients with HER2 IHC 3+ showed significantly longer OS (29 vs. 15.3 months; P = 0.025) than patients with IHC ≤ 2+. An HER2/CEP17 ratio of 4.48 was the optimal cutoff for predicting longer OS (26.9 vs. 14.7 months; P = 0.027). In subgroup analysis, treatment outcomes of patients with IHC 3+ were not influenced by the level of HER2 gene amplification. However, in patients with IHC ≤ 2+, an HER2/CEP17 ratio more than 3.69 and HER2 GCN more than 7.75 were positive predictive factors for better outcomes with trastuzumab-based chemotherapy. These findings were confirmed in both the validation cohort and the combined cohort.

Conclusions: HER2 IHC status, HER2/CEP17 ratio, and HER2 GCN were correlated with clinical outcomes of trastuzumab-based treatment in HER2-positive AGC. Clinical outcomes of patients with IHC ≤ 2+ were strongly dependent on the HER2/CEP17 ratio and HER2 GCN. Clin Cancer Res; 21(11); 2520–9. ©2015 AACR.

Introduction

Gastric cancer is the third leading cause of cancer-related deaths worldwide, and advanced cases have a median OS time of around 1 year with cytotoxic chemotherapy (1, 2). The prognosis of a subset of patients with HER2 amplification or overexpression has been improved by using anti-HER2 antibody (HER2; also known as ERBB2 or c-erbB2). The ToGA trial evaluated the addition of trastuzumab (Herceptin; F. Hoffmann-La Roche) to conventional chemotherapy in HER2-positive AGC and showed improved survival for patients treated with trastuzumab (3). The inclusion criteria of the ToGA trial was initially designed as HER2 IHC 3+ or HER2 FISH positive (HER2/CEP17 ratio ≥ 2), but preplanned analysis showed trastuzumab efficacy was demonstrated mainly in HER2 IHC 3+ or IHC 2+/FISH–positive groups. This led to controversial definitions of HER2 positivity in AGC, so the FDA granted approval to patients with IHC 3+ or FISH+, but the European Medicines Agency limited approval to patients whose tumors have IHC 3+ or IHC 2+/FISH+ (4, 5).

Although trastuzumab-based first-line treatment represents the standard approach for HER2-positive AGC, not all patients benefit from this treatment, and the overall response rate (ORR) has been variable (about 32%–68%; refs. 3, 6, 7). This implies that there is a proportion of the patients who are not responsive to trastuzumab, even though their tumors are conventionally defined as HER2-positive AGC.

On the basis of previous experience with trastuzumab treatment in breast cancer, an appropriate HER2 evaluation by IHC as well as gene amplification should be performed to identify optimal candidates for anti-HER2 therapy (8–11). However, the correlation between HER2 gene amplification and trastuzumab sensitivity has not been well evaluated in gastric cancer. A recent report suggested that the level of HER2 gene amplification was a predictive factor for sensitivity to trastuzumab-based therapy in AGC (12). Patients with an HER2/CEP17 ratio of more than 4.7 had favorable clinical outcomes. However, most...
patients were HER2 IHC 3+, and the proportion of patients with HER2 IHC equal to or less than 2+ was too small (13.6%, 9/66 patients), compared with the ToGA trial (48.0%, 141/294 in trastuzumab-treated arm) to reflect the general HER2+ AGC population.

In this study, we evaluated the association between sensitivity to trastuzumab-based chemotherapy and levels of HER2 gene amplification and compared its predictability according to HER2 IHC status in HER2-positive AGC. We hypothesized that the predictive value of HER2 gene amplification for clinical outcomes would be more evident in patients whose IHC is equal to or less than IHC 2+.

Patients and Methods

Patients

This study was a retrospective analysis of deidentified patient level data collected from medical charts. Patients diagnosed with AGC at Seoul National University Hospital, Republic of Korea, from September 2004 to March 2014, were included in the training cohort (Supplementary Fig. S1, CONSORT diagram). Men or women older than 18 years of age were included if they had histologically confirmed recurrent or metastatic AGC, an Eastern Cooperative Oncology Group performance status (ECOG PS) of 0 to 2, adequate organ function, documented HER2 status by IHC and/or FISH, and had received trastuzumab-based chemotherapy. Patients with a short duration of follow-up (less than 6 months) without confirmed death were excluded from the analysis.

A total of 126 HER2-positive AGC patients were included in the training cohort. HER2 IHC status (N = 126), HER2/CEP17 ratio (N = 66), and HER2 gene copy number (GCN; N = 59) were analyzed for clinical outcomes, including OS, progression-free survival (PFS), and ORR.

We established the independent validation cohort consisting of HER2-positive AGC patients who were treated with trastuzumab-based chemotherapy from three hospitals in Korea (Seoul National University Bundang Hospital, Seoul National University Borame Medical Center, and Seoul National University Hospital). A total of 72 patients were included in the validation cohort.

Tumor specimens and HER2 IHC/FISH

Surgically resected or endoscopically biopsied, formalin-fixed, paraffin wax-embedded tissue blocks were obtained from the archives. In each case, the pathologists examined a parallel hematoxylin and eosin stained slide to locate the areas of invasive carcinoma (13). IHC was scored according to the ToGA study criteria, which was modified on the basis of the criteria by Hofmann and colleagues (3, 14). HER2 GCN and centromere enumerator probe 17 (CEP17) were investigated by FISH using the PathVysion HER2 DNA Probe Kit (Abbott Laboratories).

The pathologists reported average copy numbers of HER2 and CEP17 in each case. We classified an HER2/CEP17 ratio more than 2.0 as positive in this study.

Statistical analysis

To assess whether increased HER2 IHC, HER2/CEP17 ratio, and HER2 GCN affect sensitivity to trastuzumab-based treatment in terms of OS, PFS, and ORR, patients were dichotomized into a good survival group (median OS longer than 12 months, median PFS longer than 6 months) versus poor survival group and into a responding group (whose best response was complete or partial remission by RECIST 1.1 criteria; ref. 15) or nonresponding group (whose best response was stable or progressive disease). The cutoff points of OS of 12 months and PFS of 6 months were approximated on the basis of general median survivals in previous reports of AGC treated with chemotherapy (2, 16) as well as a recent study (12) that had a design similar to this study. The optimal cutoff for the HER2/CEP17 ratio and HER2 GCN that discriminated between a positive or negative result in terms of response to treatment and better OS was determined using receiver operating characteristic (ROC) curve analyses. To further evaluate the performance of the HER2/CEP17 ratio and HER2 GCN in predicting sensitivity to trastuzumab-based treatment and patient survival, we calculated the AUC and compared the correlated ROC curve under nonparametric assumptions with the DeLong test (17). The $\chi^2$ test was used to determine the nature of the associations between optimal cutoff points and clinicopathologic parameters. Kaplan–Meier estimates and Cox regression analyses of OS and PFS were performed. Results were considered significant when $P$ values were less than 0.05. Analyses were done with STATA version 12 (StataCorp LP).

Ethics

The study protocol was reviewed and approved by the Institutional Review Board of Seoul National University Hospital (H-1306-007-493), Seoul National University Bundang Hospital (B-1411/276-401), and Seoul National University Borame Medical Center (16-2014-147). All studies were conducted according to guidelines (Declaration of Helsinki) for biomedical research.

Results

Patient characteristics and HER2 status

Among all 126 cases, 2 cases (1.6%) were IHC negative, 5 (4.0%) were IHC 1+, and 30 (23.8%) were IHC 2+ (Table 1). Those cases were confirmed as HER2 positive by a FISH HER2/CEP17 ratio more than 2.0. Eighty-nine (70.6%) cases were IHC 3+, and among them, 6 cases were FISH negative (Supplementary
Table S1). The representative images of HER2 IHC as well as HER2 FISH are shown in Supplementary Fig. S2. The median HER2/CEP17 ratio was 5.8 [95% CI, 1.3–12.1; range, 1.0–16.0] and median HER2 GCN was 9.9 [95% CI, 2.2–29.3; range, 2.0–40.0]. Median follow-up duration was 27.3 (range, 7.2–98.8) months. Median OS and PFS of all patients were 25.7 [95% CI, 17.5–31.2; range, 1.6–98.8] months and 8 [95% CI, 6.8–10.1; range, 0.7–85] months, respectively.

Clinical outcomes according to HER2 protein expression by IHC. Survival analysis based on HER2 IHC status was performed. The median OS of patients with IHC 3+ (n = 89) were significantly longer than that of patients with IHC 2+/FISH+ (n = 30; 29 vs. 15.7 months, respectively; HR, 0.83; 95% CI, 0.70–0.99; P = 0.038; Fig. 1A). ORR was not significantly different according to IHC status (IHC−: 50%; IHC 1+: 20%; IHC 2+: 63.3%; IHC 3+: 70.7%).
62.9%; \( P = 0.279 \); Fig. 1B). Because the median OS of IHC 1+/FISH+ (\( n = 5 \)) and IHC-/FISH+ (\( n = 2 \)) was inadequate to analyze because of the small number, we simply divided IHC 3+ and IHC 2+/FISH+ groups. The OS of the IHC 3+ group was significantly prolonged compared with the IHC 2+/FISH+ group (median OS 29 vs. 15.3 months, respectively; HR, 0.57; 95% CI, 0.35–0.93; \( P = 0.025 \); Fig. 1C), and the similar trend was observed in terms of PFS (data not shown). However, ORR of the two groups were not significantly different (56.8% vs. 62.9%, respectively; \( P = 0.518 \); Fig. 1D).

Clinical outcomes according to levels of HER2 gene amplification by FISH

For the HER2/CEP17 ratio, described as HER2 gene amplification (18), ROC analysis was performed to elucidate the optimal cutoff value to determine which patients had good clinical outcomes after trastuzumab-based treatment. The optimal cutoff of the HER2/CEP17 ratio for predicting OS ≥ 12 months was 4.48, and the area under ROC curve (AUC) was 0.664 (95% CI, 0.518–0.810; \( P = 0.025 \); Fig. 2A). The median OS of patients with HER2/CEP17 ratio ≥ 4.48 (\( n = 37 \)) was significantly longer than that of the group with a ratio < 4.48 (\( n = 29 \); 26.9 vs. 14.7 months, respectively; HR, 0.46; 95% CI, 0.23–0.91; \( P = 0.027 \); Fig. 2B). A similar trend was observed in regard to PFS (data not shown).

An HER2/CEP17 ratio of 4.21 was also the optimal cutoff for predicting objective response, and AUC of this analysis was 0.650 (95% CI, 0.497–0.804; \( P = 0.051 \); Fig. 2C), and the ORR of patients with HER2/CEP17 ratio ≥ 4.21 was 84.2% compared with 46.4% in those with a ratio < 4.21 (\( P = 0.001 \); Fig. 2D).

The impact of HER2 gene amplification on treatment outcomes according to HER2 IHC status

Because both HER2 IHC (Fig. 1) and HER2 gene amplification (Fig. 2) were positively correlated with treatment outcomes, we further conducted subgroup analysis to find out which groups of patients were more dependent on HER2 gene amplification in terms of predicting outcomes of trastuzumab-
based treatment. Figure 3A compares ROC curves predicting better OS with the HER2/CEP17 ratio in the HER2 IHC 3+ and HER2 IHC ≤ 2+ groups. The AUC for predicting better OS was 0.857 in the IHC ≤ 2+ groups (95% CI 0.687–1.00; P = 0.001); in contrast, the AUC was 0.505 in the IHC 3+ groups (95% CI, 0.293–0.717; P = 0.962, Fig. 3A). Consistent with this result, the OS was not different in patients with IHC 3+ when they were divided by the level of HER2/CEP17 ratio of 3.69 (HR, 1.06; 95% CI, 0.34–3.35; P = 0.918; Fig. 3B). However, the optimal cutoff of the HER2/CEP17 ratio 3.69 clearly demonstrated a division in OS in patients with IHC ≤ 2+ (OS of a ratio ≥ 3.69: 23.3 vs. <3.69: 7.7 months; HR, 0.21; 95% CI, 0.08–0.60; P = 0.003; Fig. 3B). A similar pattern was also observed for PFS (data not shown).

The ROC curves for predicting objective response only showed significance in the IHC ≤ 2+ group (AUC, 0.765; 95% CI, 0.588–0.962; P = 0.020; optimal cutoff 4.21), not in the IHC 3+ group (AUC, 0.548; 95% CI, 0.327–0.769; P = 0.654; Fig. 3C). ORR of HER2/CEP17 ratio ≥ 4.21 was significantly higher compared with that of a ratio < 4.21 in the IHC ≤ 2+ group (91.7% vs. 37.5%, respectively, P = 0.004), but this trend lost statistical significance in the IHC 3+ group (80.8% vs. 58.3%, respectively, P = 0.144; Fig. 3D).

We did the same analysis using HER2 GCN. The ROC analysis showed the optimal cutoffs of HER2 GCN for predicting better OS and response were 7.75 and 7.15, respectively (AUC 0.669 and 0.661; P = 0.033 and 0.044, respectively; Supplementary Fig. S3). Similar to the HER2/CEP17 ratio, the association of treatment outcomes with HER2 GCN was more definite in the IHC ≤ 2+ group compared with the IHC 3+ group (Supplementary Fig. S4).

The predictive value of HER2/CEP17 ratio according to other clinicopathologic factors

Although several clinicopathologic characteristics such as old age, liver metastasis, peritoneal metastasis, and high inflammatory markers (19–22) are known to be prognostic factors in AGC, little is known in the matter of HER2+ AGC. We conducted univariate and multivariate survival analysis by clinicopathologic characteristics (Supplementary Table S2). Multivariate analysis showed that the level of HER2/CEP17 significantly affected OS (Fig. 4).
Confirmatory analysis of HER2 IHC, HER2/CEP17 ratio, and HER2 GCN in the validation cohort

The findings from the training cohort were confirmed in the independent validation cohort. A total of 72 HER2-positive AGC patients treated with trastuzumab-based chemotherapy were recruited for the validation cohort. The median follow-up duration and OS of the validation cohort were 13.1 months (range, 0.9–95.9 months) and 33.9 months (95% CI, 19.1–36.6 months), respectively (Table 1). Patients with HER2 IHC 3+ had significantly longer OS compared with those in the HER2 IHC 2+/C20 group (median OS, 33.9 vs. 10.5 months, P = 0.0369, Fig. 5A). Moreover, patients with an HER2/CEP17 ratio greater than 4.48 showed better survival than the other patients (median OS, 36.4 vs. 19.1 months, P = 0.0369, Fig. 5A). Moreover, patients with an HER2/CEP17 ratio greater than 4.48 showed better survival than the other patients (median OS, 36.4 vs. 19.1 months, P = 0.0369, Fig. 5A). Patients with an IHC 3+ had a good prognosis irrespective of the HER2/CEP17 ratio (P = 0.0369, Supplementary Fig. S5A).

The analysis in the combined cohorts (training cohort plus validation cohort, N = 198) also confirmed that patients with an HER2 IHC 3+ (P = 0.0038), an HER2/CEP17 ratio greater than 4.48 (P = 0.0022), and an HER2 GCN greater than 7.75 (P = 0.0009) had significantly better prognosis. Moreover, in the IHC ≤ 2+ group, patients with an HER2/CEP17 ratio greater than 3.69 (P = 0.0039) and HER2 GCN greater than 7.75 (P = 0.0409) showed significantly better survival outcomes (Fig. 5D–F and Supplementary Fig. SSC and SSD).

Discussion

In this study, we showed that HER2 IHC status, HER2/CEP17 ratio, and HER2 GCN were associated with the clinical outcomes of trastuzumab-based treatment in HER2+ AGC. Patients with IHC 3+ demonstrated favorable OS, PFS, and ORR, irrespective of the HER2/CEP17 ratio and HER2 GCN. However, clinical outcomes of patients with IHC ≤ 2+ were strongly dependent on the HER2/CEP17 ratio and HER2 GCN.
In the case of breast cancer, ASCO/CAP 2013 guidelines for HER2 testing have suggested HER2 positivity to be HER2 IHC 3+ or HER2 gene amplification confirmed by either single-probe average HER2 copy number ≥ 6.0 signals per cell or dual-probe HER2/CEP17 ratio ≥ 2.0 (23). The ToGA trial used the same criteria for HER2 positivity, but post hoc analysis showed HER2 IHC 1+ or IHC-negative patients with confirmed HER2 gene amplification did not benefit by the addition of trastuzumab to cytotoxic chemotherapy. Moreover, determining the threshold of the HER2/CEP17 ratio that predicts benefit from trastuzumab therapy might be different from that of the definition of HER2 positivity in gastric cancer. Because of the limited number of studies using trastuzumab in AGC, a firm definition of HER2 positivity as well as the cutoff value for the HER2/CEP17 ratio has not been clearly established, especially in terms of predicting benefit from trastuzumab therapy.

HER2 protein overexpression, clinically determined by IHC, has been the historical predictive factor of response to trastuzumab in metastatic breast cancer (8). Although the results of ToGA showed that the OS might be prolonged in IHC 3+ compared with IHC 2+ (17.9 vs. 12.3 months), direct comparison of treatment outcomes from trastuzumab between IHC 3+ versus IHC ≤ 2+ has not been reported in AGC. In this study, we clearly showed that OS and PFS were significantly prolonged with trastuzumab in patients with IHC 3+ compared with those with IHC 2+, irrespective of HER2 FISH status. Patients with IHC 1+ in this study showed favorable clinical outcomes, but the number in this group (n = 5) was too small to analyze. In general, OS and PFS of patients with IHC 3+ were longer than in patients with IHC ≤ 2+.

The levels of HER2 gene amplification might be the predictive factor of trastuzumab treatment in HER2+ AGC. Consistent with a recent report (12), we showed patients with higher HER2 gene amplification, reflected by HER2/CEP17 ratio and HER2 GCN, had better clinical outcomes. HER2/CEP17 ratio of 4.48 was the optimal cutoff value for predicting OS longer than 12 months. This cutoff value is very similar to 4.45 in a previous report (12).

The unique findings of our study as compared with a recent report (12) is that we noted that the clinical outcomes of most patients with IHC 3+ were good irrespective of the HER2/CEP17 ratio, even in patients with an HER2/CEP17 ratio less than 2.0. This suggests that in the HER2 IHC 3+ population, the level of HER2 gene amplification does not provide additional information predictive of clinical outcomes. However, the predictive values of both the HER2/CEP17 ratio and HER2 GCN were meaningful in patients with IHC ≤ 2+, with optimal cutoff values of 3.69 and 7.75, respectively, for predicting better OS. These are
higher than the current cutoff of FISH positivity of HER2 (i.e., 2.0 and 6.0, respectively). The proportion of patients with an IHC ≤ 2+ and an HER2/CEP17 ratio < 3.69 in this study was 22.7% (15/66 patients), and the median OS of this group was 7.7 months. This was considerably worse compared with both of the other groups in our study as well as patients in the ToGA trial. Therefore,
our study provides information that is both unique and relevant to clinical outcomes.

As trials of trastuzumab on HER2-positive breast cancer (24) as well as trials of other HER2-targeting agents such as lapatinib on HER2-positive AGC demonstrate (25, 26), the appropriate usage of an HER2-targeting agent according to accurate HER2 status is important. TyTAN was a phase III trial to evaluate the efficacy of adding lapatinib to paclitaxel in previously treated HER2-amplified AGC (26). Although this trial failed to show significant improvement in OS (median OS of lapatinib vs. placebo, 11.0 vs. 8.9 months, \( P = 0.1044 \)), a subgroup analysis showed that patients with HER2 IHC 3+ benefited greatly from the addition of lapatinib compared with those with HER2 IHC 2+ or 0/1+ (median OS, 14.0 vs. 7.6 months, \( P = 0.0176 \), respectively), with a trend similar to our primary results. Therefore, defining accurate HER2-positive AGC and identifying groups that would benefit from HER2-targeting treatment are of utmost importance.

According to our results, the cutoff value of HER2/CEP17 ratio for selection of patients with HER2 IHC \( \leq 2+ \) to receive trastuzumab treatment would be considered to be 3.69, which is higher than the conventional consensus of 2.0. Moreover, on the basis of our results, in patients with IHC 3+, information from HER2 gene amplification might not influence clinical decisions regarding trastuzumab-based treatment. However, in patients with an IHC \( \leq 2+ \), further information from HER2 gene amplification status could provide the clinician with better guidance in selecting patients who might benefit from trastuzumab. When we set the cutoff for better survival as the median OS of the training cohort (25.7 months), the same result was observed (data not shown).

We also noticed better survival in our cohort compared with that of the ToGA study. As presented in Table 1, 28 patients (22.2%) received other HER2-targeting inhibitors after trastuzumab failure during clinical trials (e.g., TyTAN), and the median OS of those patients was 35 months (95% CI, 21.9–45.3), which might be one of contributing factors for the longer OS in our study. The OS of HER2-positive gastric AGC patients has rarely been reported other than in the ToGA study. Our study has value in providing additional information.

Our study has a limitation of being a retrospective analysis. However, we validated our findings with an independent validation cohort. Therefore, we believe that our investigation of the association between HER2 gene amplification and clinical outcomes is valuable.

In conclusion, HER2 IHC status, HER2/CEP17 ratio, and HER2 GCN were correlated with clinical outcomes of trastuzumab-based treatment in HER2-positive AGC. Clinical outcomes of patients with IHC \( < 2+ \) were strongly dependent on the HER2/CEP17 ratio and HER2 GCN. Further discriminatory selection of patients based on this information is helpful to make individualized medical decisions in cases of HER2-positive gastric cancer.

Disclosure of Potential Conflicts of Interest

S-A. Im is an uncompensated consultant/advisory board member for Roche. No potential conflicts of interest were disclosed by the other authors.

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